Greenwoodochromini Takahashi from Lake Tanganyika is a junior synonym of Limnochromini poll (Perciformes: Cichlidae).

AUTHOR(S):

Takahashi, T

Greenwoodochromini Takahashi from Lake Tanganyika is a junior synonym of Limnochromini Poll (Perciformes: Cichlidae)

T. Takahashi

Laboratory of Animal Ecology, Graduate School of Science, Kyoto University, Kitashirakawa-Oiwake, Sakyo, Kyoto 606-8502, Japan

Running headline: SYNONYM OF A LAKE TANGANYIKA CICHLID TRIBE

Tel: +81 75 753 4077; fax: + 81 75 753 4100; e-mail: tetsumi@terra.zool.kyoto-u.ac.jp

The infraorbitals (IOs) of four species endemic to Lake Tanganyika were examined. Based on the examination of the IOs and previous morphological and molecular studies, the tribe Greenwoodochromini is synonymised with the tribe Limnochromini, and a new combination for *Limnochromis abeelei* and *Limnochromis staneri* is proposed: *Greenwoodochromis abeelei* and *Greenwoodochromis staneri*. The revised tribe Limnochromini, which consists of ten species belonging to seven genera, is characterised by IOs representing types G and I, and the revised genus *Greenwoodochromis*, which consists of four species, is characterised by IOs representing type I.

Key words: infraorbitals; tribes; genus; classification; taxonomy.

INTRODUCTION

In Lake Tanganyika, about 200 endemic cichlid species are currently considered valid, and new species continue to be described (*e.g.*, Takahashi & Hori, 2006; Schelly *et al.*, 2007; Verburg & Bills, 2007; Takahashi, 2008; Burgess, 2012; Kullander *et al.*, 2012). These fishes are morphologically, ecologically and genetically diverse, and represent a well-established model system for the study of adaptive radiation (*e.g.*, Fryer & Iles, 1972; Kornfield & Smith, 2000; Turner *et al.*, 2001; Kocher, 2004; Seehausen, 2006; Turner, 2007; Koblmüller *et al.*, 2008; Salzburger, 2009; Sturmbauer *et al.*, 2011; Takahashi & Koblmüller, 2011; Gante & Salzburger, 2012).

Poll (1986) first classified the Lake Tanganyika cichlid fishes into 12 tribes based on morphological features. Some molecular and morphological studies pointed out that this classification was essentially reasonable but needed a few minor changes (Nishida, 1991; Kocher *et al.*, 1995; Lippitsch, 1998; Salzburger *et al.*, 2002). Subsequently, Takahashi (2003*a*) constructed a cladogram of these fishes based on anatomical data and proposed a new classification that recognised 16 tribes. Takahashi's (2003*a*) classification resolved some taxonomic problems with Poll's (1986) classification, but some molecular phylogenetic studies have highlighted the need for further minor changes (reviewed by Koblmüller *et al.*, 2008). One of the contradictions between Takahashi's (2003*a*) classification and molecular phylogenetic studies raised a problem on the validity of the tribe Greenwoodochromini Takahashi, 2003 (Duftner *et al.*, 2005).

In Takahashi's (2003*a*) classification, the genus *Greenwoodochromis* Poll, 1986 was

61 isolated from the tribe Limnochromini Poll, 1986 and Greenwoodochromini was
62 established for this genus [at the same time, Takahashi (2003*a*) also isolated the genus
63 *Benthochromis* Poll, 1986 from Limnochromini and established the tribe
64 Benthochromini Takahashi, 2003 for this genus, and moved *Gnathochromis pfefferi*
65 (Boulenger, 1898) from the Limnochromini to the Tropheini Poll, 1986, although
66 *Gnathochromis permaxillaris* (David, 1936), which is the type species of
67 *Gnathochromis* Poll, 1981, remained in Limnochromini]. However, the
68 Greenwoodochromini is nested within Takahashi's (2003*a*) Limnochromini in a
69 molecular phylogeny resulting in a polyphyletic Limnochromini (Duftner *et al.*, 2005;
70 and see Discussion).
71
72 Takahashi (2003*a*) used a morphological difference in the infraorbitals (IOs), which are
73 bones surrounding the lower half of the eye, to distinguish the Greenwoodochromini
74 from the Limnochromini. The IO series of the type species of *Greenwoodochromis*,
75 *Greenwoodochromis christyi* (Trewavas, 1953), were identified as type A (according to
76 the definitions of Takahashi, 2003*b*), which is the most common of the Lake Tanganyika
77 cichlid flock; whereas the IOs of species of Limnochromini were identified as type G
78 [Fig. 1(a); Takahashi, 2003*a*, *b*]. However, the number of specimens of *G. christyi* used
79 by Takahashi (2003*a*, *b*) was small, and IOs from *Greenwoodochromis bellcrossi* (Poll,
80 1976), *Limnochromis abeelei* Poll, 1949, and *Limnochromis staneri* Poll, 1949 were not
81 examined. Although few samples are available because of the difficulties in collecting
82 from deep-water habitats, the present study examined the IO series of six to 20
83 specimens from these four species. By combining the present anatomical data with
84 previously published molecular phylogenetic and morphological studies, the validity of

85 Greenwoodochromini and the extent of *Greenwoodochromis* are discussed.

86

87

88 **MATERIALS AND METHODS**

89

90 *Greenwoodochromis christyi*, *G. bellcrossi*, *L. abeelei*, and *L. staneri* were collected off
91 Mtondwe Island, Mpulungu, Zambia, with gill nets at 42–140 m depth between August
92 1995 and December 2007 and in October 2013 (N = 57). Fish were fixed in 10%
93 formalin. The standard length (L_S) of each fish was measured with digital callipers. The
94 IOs on the left side of the head were observed. The IOs of some specimens were
95 removed from the head and stained with Alizarin Red-S in 70% ethyl alcohol, and
96 sketches were made under a binocular microscope (Nikon SMZ 1000). A likelihood
97 ratio test on a logistic regression was used to test any association between intra-specific
98 variation in IOs and fish size. The term "anteriormost bone" is used instead of lacrimal.

99

100

101 **RESULTS**

102

103 The number of bones in the IO series ranges from four to six in *G. christyi*, *G. bellcrossi*,
104 *L. abeelei*, and *L. staneri* (Table I). The anteriormost bone is larger than the others and
105 has a sensory canal that opens through five or six sensory pores. Four of the pores are
106 large; the widths of these pores are wider than the intervals between the pores [arrows in
107 Fig. 2(a-c)]. The remaining small, tube-like bones have two to five (usually two)
108 sensory pores each.

Greenwoodochromis christyi has two major variations in IO configuration (Table I). Thirteen out of the 20 individuals examined have six IOs with 6-2-2-2-2-2 sensory pores [Fig. 2(b)], whereas five individuals have five IOs with 6-2-3-2-2 sensory pores [Fig. 2(a)]. The third bone of the latter variation (6-2-3-2-2) is elongated and has three sensory pores; two pores are located at the ends of the bone, and the other pore is located anterior to the midpoint of the bone. The individuals that have five IOs with 6-2-3-2-2 sensory pores are significantly larger than the individuals that have six IOs with 6-2-2-2-2-2 sensory pores (Fig. 3).

In *G. bellcrossi* and *L. abeelei*, the IO series varies greatly in numbers of bones and sensory pores, although smaller numbers of samples were examined compared to the other species (Table I). The major variation accords with the IO configuration of the large individuals of *G. christyi* (6-2-3-2-2). One individual of *L. abeelei* exhibits the same configuration as the small individuals of *G. christyi* (6-2-2-2-2-2).

In *L. staneri*, the IO series varies somewhat in numbers of bones and sensory pores (Table I). Fourteen of the 20 individuals examined have six bones with 5-2-2-3-2-2 sensory pores [Fig. 2(c)]. The fourth bone is elongate and has a pore located at each end, and the other pore is located anterior to the midpoint of the bone. One individual exhibits the same condition as the small individuals of *G. christyi* (6-2-2-2-2-2).

DISCUSSION

The IOs of *G. christyi*, *G. bellcrossi*, *L. abeelei*, and *L. staneri* are distinguishable from those of the other Lake Tanganyika cichlids. In these species, four of the five or six sensory pores on the anteriormost bone are large and the widths of these four pores are wider than the intervals between the pores [Fig. 2(a-c)], whereas the widths of all the sensory pores on the anteriormost bone are small and the widths of the pores are narrower than the intervals between the pores in many other species (Takahashi, 2003*b*). Species of *Trematocara* Boulenger, 1899 also have large pores on the anteriormost bone, but all the pores (not only four of the pores) are large (Takahashi, 2002, 2003*b*).

Several variations were found in IO configuration of *G. christyi*, *G. bellcrossi*, *L. abeelei*, and *L. staneri* (Table I). Taking into account the size and number of sensory pores on the bones and distances between the pores, these variations may be a result of a few fusion events between certain bones. For example, the anteriormost and second bones of *L. staneri* [Fig. 2(c)] appear to be homologous with the anteriormost bone of *G. christyi* [Fig. 2(a, b)], and the third and fourth bones of small individuals of *G. christyi* [Fig. 2(b)] appear to be homologous with the third bone of the large individuals of *G. christyi* [Fig. 2(a)] and the fourth bone of *L. staneri* [Fig. 2(c)]. Accordingly, assuming a plesiomorphic IO configuration of 5-2-2-2-2-2-2, the most common condition of small individuals of *G. christyi* (6-2-2-2-2-2) can be considered a result of fusion between the first and second bones of the plesiomoprhic IO configuration [Fig. 2(b)]. The most common condition of large individuals of *G. christyi* (6-2-3-2-2), which is also the most common condition of *G. bellcrossi* and *L. abeelei*, can be considered a result of fusion between the first and second bones and between the fourth and fifth bones of the

157 plesiomorphic IO configuration [Fig. 2(a)]. Similarly, the most common condition of *L.*
158 *staneri* (5-2-2-3-2-2) can be considered a result of fusion between the fourth and fifth
159 bones of the plesiomorphic IO configuration [Fig. 2(c)]. Other minor variations can be
160 explained by an irregular increase in the number of sensory pores on a tube-like bone,
161 an increase or decrease of the number of tube-like bones, or unusual fusion event (see
162 Table I foot notes).
163
164 Takahashi (2003*b*) described IOs of Lake Tanganyika cichlids and classified them into
165 eight types: types A to H. Accordingly, the condition of the IOs unique to *G. christyi*, *G.*
166 *bellcrossi*, *L. abeelei*, and *L. staneri* is named 'type I', the ninth type. Type I can be
167 defined as IOs that have four large sensory pores on the anteriormost bone, and usually
168 consist of seven IOs with 5-2-2-2-2-2-2 sensory pores, of which the first and second IOs
169 and/or the fourth and fifth IOs are fused. Takahashi (2003*b*) considered the IOs of small
170 individuals of *G. christyi* (6-2-2-2-2-2) to be a variation of type A [Fig. 2(e) shows the
171 typical condition of type A, which has six bones with 5-2-2-2-2-2 sensory pores and the
172 sensory pores on the anteriormost bone are small]; however, the 6-2-2-2-2-2 condition is
173 regarded as type I in the present study.
174
175 In their phylogenetic analysis of Lake Tanganyika cichlids using mitochondrial DNA
176 (mtDNA) sequences, Duftner *et al.* (2005) inferred that nine of the ten species in
177 Takahashi's (2003*a*) Greenwoodochromini and Limnochromini formed a monophyletic
178 group [Fig. 1(a)] (*Tangachromis dhanisi* (Poll, 1949) was not examined]. The
179 monophyly of this group is strongly supported by various statistical tests (*e.g.*, 100%
180 Bayesian posterior probability). A clade of Takahashi's (2003*a*) Greenwoodochromini

nested within the Limnochromini resulted in the Limnochromini and the genus *Limnochromis* Regan 1920 [the type species is *Limnochromis auritus* (Boulenger, 1901)] being polyphyletic [Fig. 1(a)]. Although phylogenetic inference based on mtDNA can be heavily affected by incomplete lineage sorting and introgression (*e.g.*, Rüber *et al.*, 2001; Koblmüller *et al.*, 2007*a*, *b*, 2010; Sturmbauer *et al.*, 2010), the morphology of the IOs supports the monophyly of a clade including *G. christyi*, *G. bellcrossi*, *L. abeelei* and *L. staneri* [Fig. 1(b)], which is supported by 100% Bayesian posterior probability in the mtDNA tree (Duftner *et al.*, 2005). To resolve the disagreements between systematic classification and phylogeny, it is proposed to synonymise Greenwoodochromini with Limnochromini and to make two new combinations: *Greenwoodochromis abeelei* and *Greenwoodochromis staneri* [Fig. 1(b)].

The IOs representing type G, which consists typically of three bones with the elongated second bone [Fig. 2(d); Takahashi, 2003*b*], and type I are unique to the revised Limnochromini. The species of Limnochromini inhabit bottoms at depths greater than 30 m (Poll, 1956). The large sensory pores on the anteriormost bone (type I) may possibly improve noise sensitivity in deep, dark environments, and fusion of bones (type G, type I) may possibly strengthen the structure.

In conclusion, the tribe Greenwoodochromini is synonymised with the tribe Limnochromini. The revised Limnochromini consists of seven genera: *Baileychromis*, *Gnathochromis* (excluding *G. pfefferi*, which is included in the tribe Tropheini), *Greenwoodochromis*, *Limnochromis*, *Reganochromis*, *Triglachromis* and presumably *Tangachromis* (although it's phylogenetic position has not been directly examined here

of by Duftner *et al.*, 2005). This Limnochromini is characterised by having the IOs representing type G [Fig. 2(d)] and type I [Fig. 2(a-c)]. The genus *Greenwoodochromis* is revised to include four species: *G. abeelei* (new combination), *G. bellcrossi*, *G. christyi* (type species of this genus), and *G. staneri* (new combination). This genus is characterised by having IOs representing type I.

Acknowledgements. I thank M. Hori for providing samples for the morphological analyses and E. Nakajima for providing language help. This study was supported by Grants-in-Aid for Young Scientists (No. 20770065) and Scientific Research (No. 23370043) of MEXT, Japan, and Global COE Program (A06) of Kyoto University.

References

Burgess, W. E. (2012). A new species of goby cichlid of the genus *Eretmodus*, *E. marksmithi*, (Pisces: Cichlidae) from the northern part of Lake Tanganyika. *Tanganika Magazyn* **12,** 23–31.

Duftner, N., Koblmüller, S. & Sturmbauer, C. (2005). Evolutionary relationships of the Limnochromini, a tribe of benthic deepwater cichlid fish endemic to Lake Tanganyika, East Africa. *Journal of Molecular Evolution* **60,** 277–289. doi: 10.1007/s00239-004-0017-8

Fryer, G. & Iles, T. D. (1972). *The cichlid fishes of the great lakes of Africa.* Neptune City, NJ: T.F.H. Publications.

Gante, H.F. & Salzburger, W. (2012). Evolution: Cichlid models on the runaway to

speciation. *Current Biology* **22,** R956–958. doi: 10.1016/j.cub.2012.09.045

Koblmüller, S., Duftner, N., Sefc, K. M., Aibara, M., Stipacek, M., Blanc, M., Egger, B. & Sturmbauer, C. (2007*a*). Reticulate phylogeny of gastropod-shell-breeding cichlids from Lake Tanganyika – the result of repeated introgressive hybridization. *BMC Evolutionary Biology* **7,** 7. doi: 10.1186/1471-2148-7-7

Koblmüller, S., Egger, B., Sefc, K. M. & Sturmbauer, C. (2007*b*). Evolutionary history of Lake Tanganyika's scale-eating cichlid fishes. *Molecular Phylogenetics and Evolution* **44,** 1295–1305. doi: 10.1016/j.ympev.2007.02.010

Koblmüller, S., Sefc, K. M. & Sturmbauer, C. (2008). The Lake Tanganyika cichlid species assemblage: recent advances in molecular phylogenetics. *Hydrobiologia* **615,** 5–20. doi: 10.1007/s10750-008-9552-4

Koblmüller, S., Egger, B., Sturmbauer, C. & Sefc, K. M. (2010). Rapid radiation, ancient incomplete lineage sorting and ancient hybridization in the endemic Lake Tanganyika cichlid tribe Tropheini. *Molecular Phylogenetics and Evolution* **55,** 318–334. doi: 10.1016/j.ympev.2009.09.032

Kocher, T. D. (2004). Adaptive evolution and explosive speciation: the cichlid fish model. *Nature Reviews Genetics* **5,** 288–298. doi: 10.1038/nrg1316

Kocher, T. D., Conroy, J. A., McKaye, K. R., Stauffer, J. R. & Lockwood, S. F. (1995). Evolution of NADH dehydrogenase subunit 2 in east African cichlid fish. *Molecular Phylogenetics and Evolution* **4,** 420–432.

Kornfield, I. & Smith, P.F. (2000). African cichlid fishes: model systems for evolutionary biology. *Annual Review of Ecology and Systematics* **31,** 163–196.

Kullander, S. O., Karlsson, M. & Karlsson, M. (2012). *Lepidiolamprologus kamambae*, a new species of cichlid fish (Teleostei: Cichlidae) from Lake Tanganyika. *Zootaxa*

3492, 30–48.

Lippitsch, E. (1998). Phylogenetic study of cichlid fishes in Lake Tanganyika: a lepidological approach. *Journal of Fish Biology* **53,** 752–766.

Nishida, M. (1991). Lake Tanganyika as an evolutionary reservoir of old Lineages of East African cichlid fishes: inferences from allozyme data. *Experientia* **47,** 974–979.

Poll, M. (1956). Poissons Cichlidae. *Résultats Scientifiques Exploration Hydrobiologique du Lac Tanganika (1946–1947). Institut Royal des Sciences Naturelles de Belgique* **3(5B),** 1–619.

Poll, M. (1986). Classification des Cichlidae du lac Tanganika. Tribus, genres et espèces. *Académie Royale de Belgique, Mémoires de la Classe des Sciences* **45,** 1–163.

Rüber, L., Meyer, A., Sturmbauer, C. & Verheyen, E. (2001). Population structure in two sympatric species of the Lake Tanganyika cichlid tribe Eretmodini: evidence for introgression. *Molecular Ecology* **10,** 1207–1225.

Salzburger, W. (2009). The interaction of sexually and naturally selected traits in the adaptive radiations of cichlid fishes. *Molecular Ecology* **18,** 169–185. doi: 10.1111/j.1365-294X.2008.03981.x

Salzburger, W., Meyer, A., Baric, S., Verheyen, E. & Sturmbauer, C. (2002). Phylogeny of the Lake Tanganyika cichlid species flock and its relationship to the central and East African haplochromine cichlid fish faunas. *Systematic Biology* **51,** 113–135.

Schelly, R., Takahashi, T., Bills, R. & Hori, M. (2007). The first case of aggressive mimicry among lamprologines in a new species of *Lepidiolamprologus* (Perciformes: Cichlidae) from Lake Tanganyika. *Zootaxa* **1638,** 39–49.

Seehausen, O. (2006). African cichlid fish: a model system in adaptive radiation

277 research. *Proceedings of the Royal Society of London B* **273,** 1987–1998. doi:
278 10.1098/rspb.2006.3539
279 Sturmbauer, C., Salzburger, W., Duftner, N., Schelly, R. & Koblmüller, S. (2010).
280 Evolutionary history of the Lake Tanganyika cichlid tribe Lamprologini (Teleostei:
281 Perciformes) derived from mitochondrial and nuclear DNA data. *Molecular*
282 *Phylogenetics and Evolution* **57,** 266–284. doi: :10.1016/j.ympev.2010.06.018
283 Sturmbauer, C., Husemann, M. & Danley, P. D. (2011). Explosive speciation and
284 adaptive radiation of East African cichlid fishes. *Biodiversity Hotspots* **2011,** 333–
285 362. doi: 10.1007/978-3-642-20992-5_18
286 Takahashi, T. (2002). Systematics of the tribe Trematocarini (Perciformes: Cichlidae)
287 from Lake Tanganyika, Africa. *Ichthyological Research* **49,** 253–259.
288 Takahashi, T. (2003*a*). Systematics of Tanganyikan cichlid fishes (Teleostei:
289 Perciformes). *Ichthyological Research* **50,** 367–382. doi:
290 10.1007/s10228-003-0181-7
291 Takahashi, T. (2003*b*). Comparative osteology of the infraorbitals in cichlid fishes
292 (Teleostei: Perciformes) from Lake Tanganyika. *Species Diversity* **8,** 1–26.
293 Takahashi, T. (2008). Description of a new cichlid fish species of the genus
294 *Benthochromis* (Perciformes: Cichlidae) from Lake Tanganyika. *Journal of Fish*
295 *Biology* **72,** 603–613. doi: 10.1111/j.1095-8649.2007.01727.x
296 Takahashi, T. & Hori, M. (2006). Description of a new Lake Tanganyikan cichlid fish of
297 the genus *Cyprichromis* (Perciformes: Cichlidae) with a note on sexual
298 dimorphism. *Journal of Fish Biology* **68,** 174–192. doi:
299 10.1111/j.1095-8649.2006.01055.x
300 Takahashi, T. & Koblmüller, S. (2011). The adaptive radiation of cichlid fish in Lake

301 Tanganyika: a morphological perspective. *International Journal of Evolutionary*
302 *Biology* **2011,** article ID 620754. doi: 10.4061/2011/620754
303 Turner, G. F. (2007). Adaptive radiation of cichlid fish. *Current Biology* **17,** R827–
304 R831.
305 Turner, G. F., Seehausen, O., Knight, M. E., Allender, C. J. & Robinson, R. L. (2001).
306 How many species of cichlid fishes are there in African lakes? *Molecular Ecology*
307 **10,** 793–806.
308 Verburg, P. & Bills, R. (2007). Two new sympatric species of *Neolamprologus*
309 (Teleostei: Cichlidae) from Lake Tanganyika, East Africa. *Zootaxa* **1612,** 25–44.

Figure legends

FIG. 1. Schematic molecular phylogeny of nine of the ten species composing Takahashi's (2003*a*) Limnochromini and Greenwoodochromini presented by Duftner *et al.* (2005). Phylogenetic position of *Tangachromis dhanisi* remains unknown. Letters on the right of the scientific names indicate types of infraorbitals. Boxes indicate tribal classification. (a) The infraorbital types (Takahashi, 2003*b*), generic classification (Poll, 1986), and tribal classification (Takahashi, 2003*a*) of previous studies. (b) The infraorbital types, generic classification, and tribal classification according to the present study.

FIG. 2. Infraorbital (IO) series on the left side of the head representing (a-c) type I, (d) type G and (e) type A. The bones are numbered from anterior to posterior (figures above the bones). Arrows indicate four large sensory pores on the anteriormost bone. Characters connected by pluses under the bones indicate suggested fusion patterns of IOs (see text for details). (a, b) *Greenwoodochromis christyi* (110.6 mm L_S and 70.3 mm L_S, respectively), (c) *Limnochromis staneri* (130.1 mm L_S), (d) *Limnochromis auritus* (114.4 mm L_S), and (e) *Simochromis diagramma* (Günther 1894) (130.0 mm L_S). Bars indicate 5 mm.

FIG. 3. Relationship between body size and configuration of IOs (six bones with 6-2-2-2-2-2 sensory pores or five bones with 6-2-3-2-2 sensory pores) in *Greenwoodochromis christyi*.

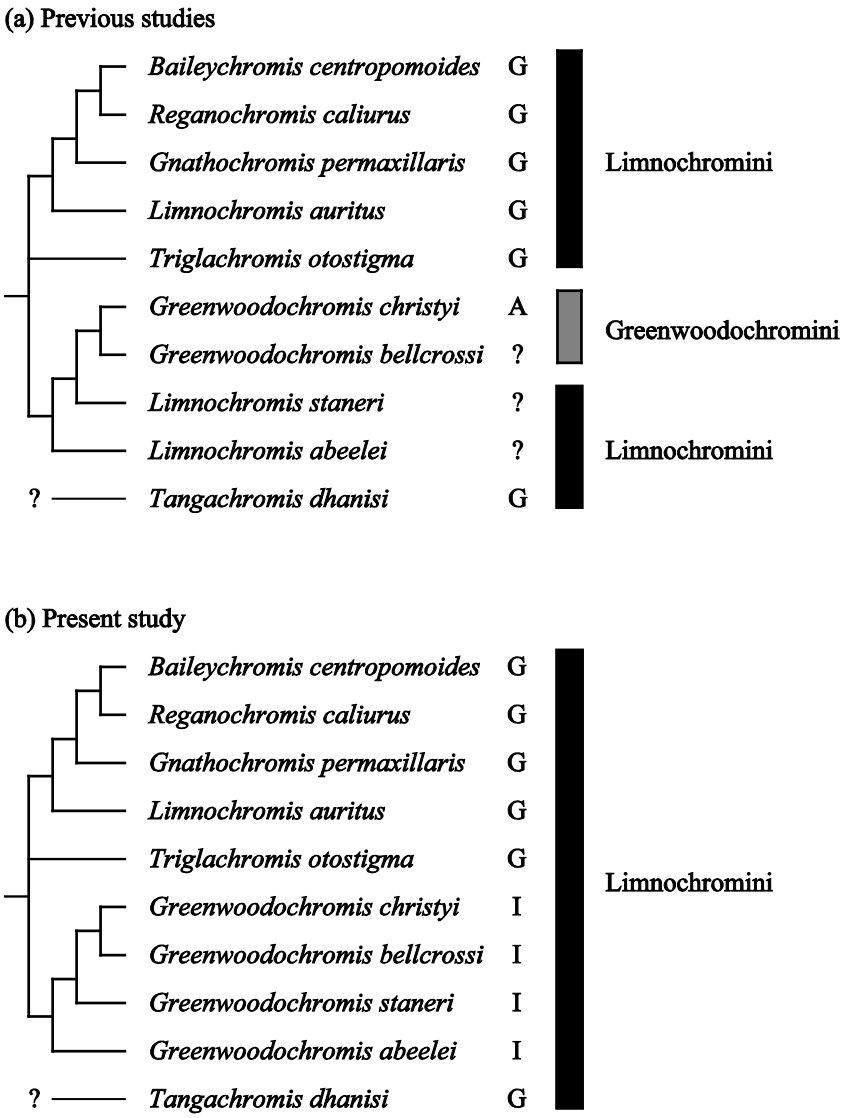

Fig. 1

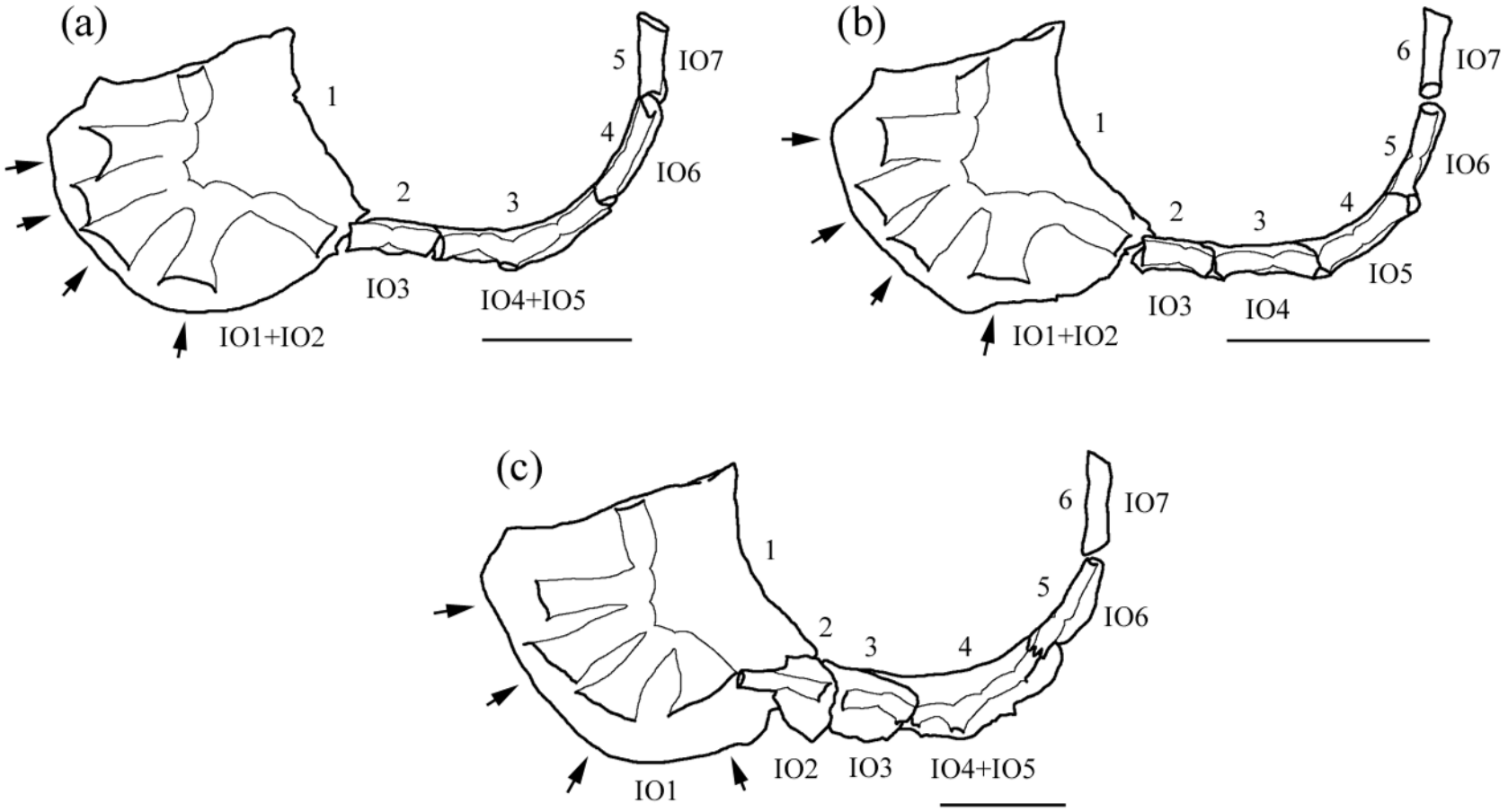
(a)
1
2
3
4
5
IO7
IO6
IO3
IO4+IO5
IO1+IO2
(b)
1
2
3
4
5
6
IO7
IO6
IO5
IO3
IO4
IO1+IO2
(c)
1
2
3
4
5
6
IO7
IO6
IO1
IO2
IO3
IO4+IO5
(d)
1
2
3
(e)
1
2
3
4
5
6

Fig. 2

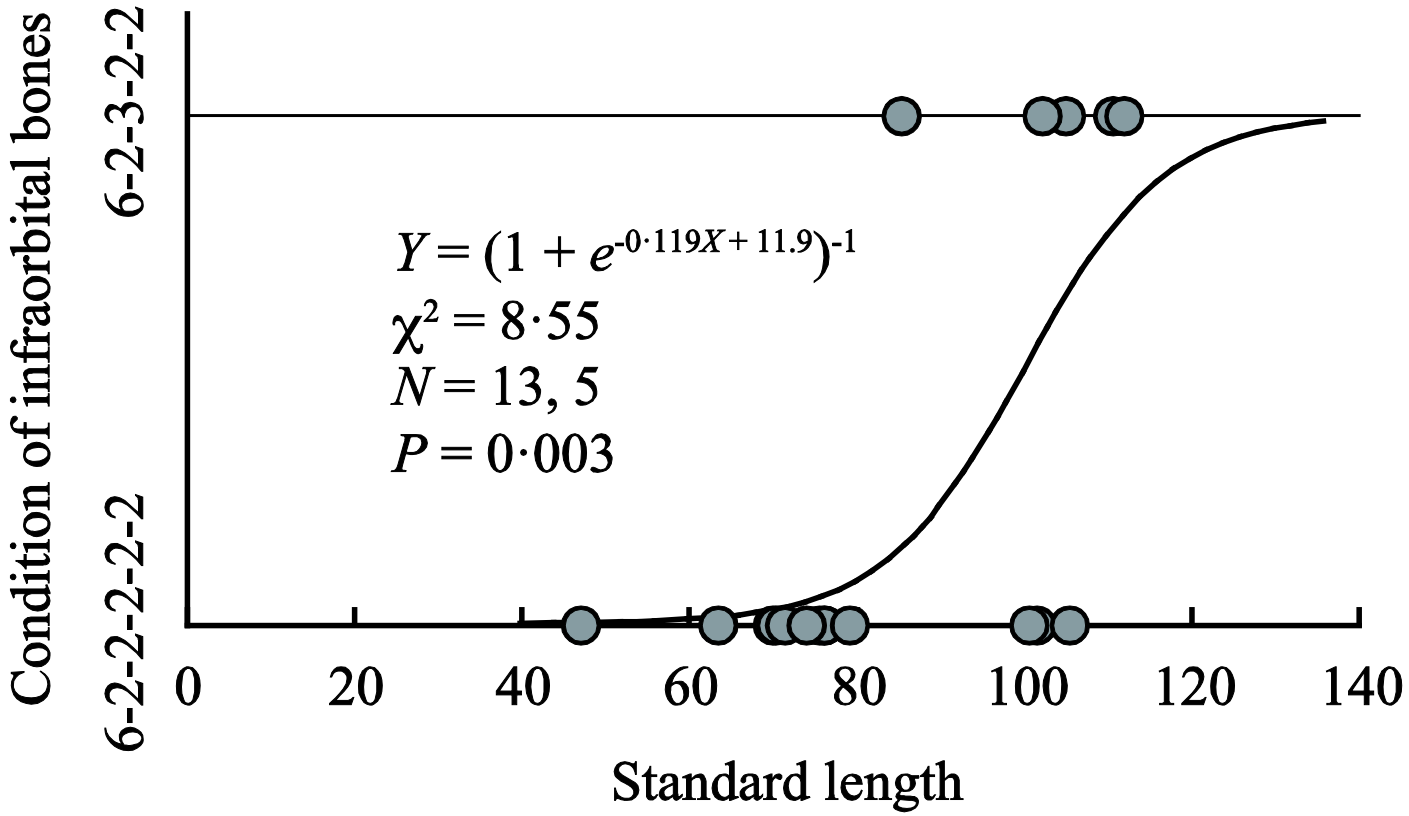

Fig. 3